

Minireview

Rab cascades and tethering factors in the endomembrane system

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Abstract Rab GTPases are key proteins that determine organelle identity and operate at the center of fusion reactions. Like Ras, they act as switches that are connected to a diverse network of tethering factors, exchange factors and GTPase activating proteins. Recent studies suggest that Rabs are linked to each other via their effectors, thus coordinating protein transport in the endomembrane system. Within this review, we will focus on selected examples that highlight these issues.

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1. Introduction

Protein transport in the endomembrane system is determined by coordinated budding and fusion events at organelle membranes. During budding, vesicles are formed at the donor organelle to take up cargo (proteins and lipids) and are consumed during fusion with the target membrane. Due to constant flux of membrane material, organelles of the endomembrane system are under a permanent threat of losing their identity as uncontrolled budding can result in complete fragmentation [1,2]. As a consequence, the fusion and fission machineries must be coordinated at each organelle and between organelles. Coordination can occur by coupling efficient budding to incorporation of the fusion machinery or by linking consecutive fusion events to each other.

Fusion requires conserved proteins that act in a regulated cascade, leading to lipid bilayer mixing. A key role in the coordination is fulfilled by the switch-like Rab GTPases in the process of vesicle fusion [3–6]. Rab-GTP binds to long or short range tethers to mediate the initial recognition of the vesicle [7]. Tethers then seem to coordinate with SNAREs on vesicle and target membrane to allow SNARE assembly *in trans* in the process of bilayer mixing [8,9]. Within this review, we will focus on the role of Rab-GTPases and tethering factors in coordinating fusion events, and will discuss their function in the context of selected examples from yeast and mammalian cells (Figs. 1 and 2).

1.1. Rabs

Despite being considered GTPases, Rabs and their colleagues of the Ras, Rho, or Ran families are incomplete enzymes; they are comparably slow in exchanging nucleotides or hydrolyzing them. These apparent shortcomings of the Rabs are in fact prerequisites to act as switches between the inactive GDP to the active GTP form, linking Rabs to an activation–inactivation cascade [3,4]. Within the cytosol, the inactive GDP-form is bound to GDI (GDP dissociation inhibitor), which also shields the Rab's hydrophobic prenyl anchor [10,11]. To bind to membranes, GDI must be displaced from Rab-GDP by a membrane bound GDF (GDI displacement factor) [12]. A number of integral membrane proteins of the Yip family have been identified as GDFs in this context. However, delivery of Rab-GDP will be a reversible process in the presence of GDI, unless an exchange factor (GEF, Guanine nucleotide exchange factor) converts the Rab into the GTP form [5,6]. The Rab-GTP can then bind to effector proteins like tethers and can thus promote vesicle binding and fusion. The Rab-cycle is completed by stimulating the GTPase of the Rab with the help of a GTP-hydrolysis activating protein (GAP), and subsequent binding of Rab-GDP binding to GDI. In sum, GEFs activate Rabs, followed by effector binding and inactivation by GAPs. Intriguingly, these distinct activities seem to be tightly coupled along the endomembrane system, and some examples are discussed in this review.

1.2. Tethers

Rabs bind to many types of effectors; we focus here on their key interaction with tethering factors. We distinguish two classes of tethers, which bind to Rabs in their GTP form: long range tethers like EEA1 [13] and p115/Usol [14] and short range, multi-subunit tethering complexes, which we will focus on below. A number of tethering complexes, which operate at different organelles, have been identified over the last years. The COG complex operates at the Golgi [15], the GARP complex is required for endosome-Golgi transport [16], the exocyst at the Golgi-plasma membrane interface [17] and the Class C-Vps/HOPS complex in the late endocytic pathway [18,19]. All complexes consist of multiple (>3) subunits with limited sequence identity [7]. However, structural analysis of some exocyst subunits revealed that the subunits share similar folds, which might be the basis for functional assembly during tethering [20]. Two additional complexes that seemingly do not bind Rab-GTP also fall into the tethering complex family: the Dsl complex, which operates between Golgi and ER [21], and the TRAPP complex required for Golgi biogenesis [22]. For the Dsl complex, a corresponding Rab has not been

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	cis-GOLGI		trans-Golgi		PLASMA MEMBRANE	
	Mammals	Yeast	Mammals	Yeast	Mammals	Yeast
Rab GTPase	Rab1	Ypt1	Rab11	Ypt31/32		Sec4
GEF	TRAPP I	TRAPP I	(TRAPP II)	TRAPP II		Sec2
EFFECTOR	p115	Uso1		Sec2	Exocyst	Exocyst

	EARLY ENDOSOME		ENDOSOME		LYSOSOME / VACUOLE	
	Mammals	Yeast	Mammals	Yeast	Mammals	Yeast
Rab GTPase	Rab5	Vps21	Rab5	Vps21	Rab7	Ypt7
GEF	Rabex5	Vps9	hVps39*		(hVps39)	Vps39
EFFECTOR	Rabaptin5 Rabenosyn5 EEA1 hVps34	Vac1	HOPS / Class C	Class C Vps8	HOPS / Class C	HOPS / Class C

Fig. 1. Selected Rabs, GEFs and effector of the secretory and endocytic pathway. Brackets on TRAPP II and hVps39 indicate that the interaction has not been formally shown. The asterisk on hVps39 indicates that binding to the GDP form, but not GEF activity has been shown [49]. Details are discussed in the text.

identified, and TRAPP appears to be a large GEF, which cooperates with other proteins during tethering [23] (see below). We will now discuss two cascades of the secretory pathway and the endo-lysosomal system, in which Rabs and tethers seem to be coupled to the maturation of the organelle (for overview see Fig. 1). We believe that with the growing insight into these systems our understanding of organelle identity in eukaryotic cells will improve decisively.

2. The Rab–tether network of the secretory pathway

Vesicular traffic from the ER to the plasma membrane is controlled by several Rab proteins. In yeast, the Ypt1 protein (a homolog of human Rab1) functions at the ER-to-*cis* Golgi and *cis*-to-medial Golgi trafficking step, Ypt31 and its homolog Ypt32 (both homologs of human Rab11) are important for the exit from the Golgi apparatus, and the Sec4 protein controls the traffic of vesicles from the *trans*-Golgi network (TGN) to the plasma membrane. Interestingly, it seems that these Rab proteins act in an organized manner, in which one activated Rab is recruiting the GEF for the Rab of the next trafficking step. Below, we will describe the current evidence for such a Rab cascade and the links to tethering complexes in the early secretory pathway, focusing primarily on the yeast system.

2.1. ER to the trans-Golgi network

At the ER–Golgi interface, COPII coated vesicles bud from the ER and fuse with the Golgi. This process seems to occur into two steps: homotypic fusion of ER vesicle to form the vesicular tubular cluster (VTC), followed by fusion of VTC clusters with the *cis*-Golgi. The Rab GTPase operating in this process, Rab1/Ypt1, is present on vesicles and binds in its GTP-form to several effectors, including the long tether Uso1 and the COG complex [24,25]. At the Golgi, Ypt1 is acti-

vated by a multi-subunit GEF complex called TRAPP complex [26]. Uso1/p115 is a large cytosolic protein that functions as a tethering factor at ER-to-Golgi step and could capture vesicles due to its extended coiled-coil domain [14,27]. A model for tethering ER-derived vesicles to the Golgi membrane in mammalian cells was lately proposed by the Sacher lab [28]. Their model assumes that the Ypt1/Rab1 activation/inactivation cycles must happen at least twice during the whole tethering process. In the first step, p115 interacts in a Rab1/Ypt1-GTP-dependent manner with vesicles that bud from the ER [29]. Ypt1/Rab1 is not in contact with its known GEF TRAPP I at that stage, indicating that additional Ypt1 GEFs must exist that is present during COPII dependent budding or on vesicles. Inactivation of Ypt1/Rab1 on vesicles could lead to conformational change of p115 and would enable the first contact of the vesicle with the membrane that is accompanied by long tethers: p115 on the vesicle and GM130 at the Golgi membrane [27,30]. At this point TRAPP I may also take part in tethering of the vesicle by binding a yet unidentified TRAPP I binding factor at its surface. However, TRAPP does not bind Ypt1/Rab1 in its GTP form [23]. Its primary role would be a (second) activation of Ypt1 at the Golgi, which may occur only once the vesicle is in close proximity to the membrane. One obvious candidate for TRAPP-dependent tethering would be the COG complex, which has been identified as a Ypt1 effector [25]. The COG complex is composed of eight subunits and is discussed primarily as a tethering complex for retrograde transport from the endosome to the *cis*-Golgi [15,31]. Its function may also include tethering of vesicles at the *cis*-Golgi, since it is required for ER to Golgi transport in vivo and in vitro [32,33].

The TRAPP complex was also shown to be a GEF for the Ypt31 and Ypt32 (Ypt31/32) Rab GTPases [23]. Interestingly, TRAPP subunits are found in two different complexes, named TRAPP I and TRAPP II [34]. TRAPP I is composed of eight

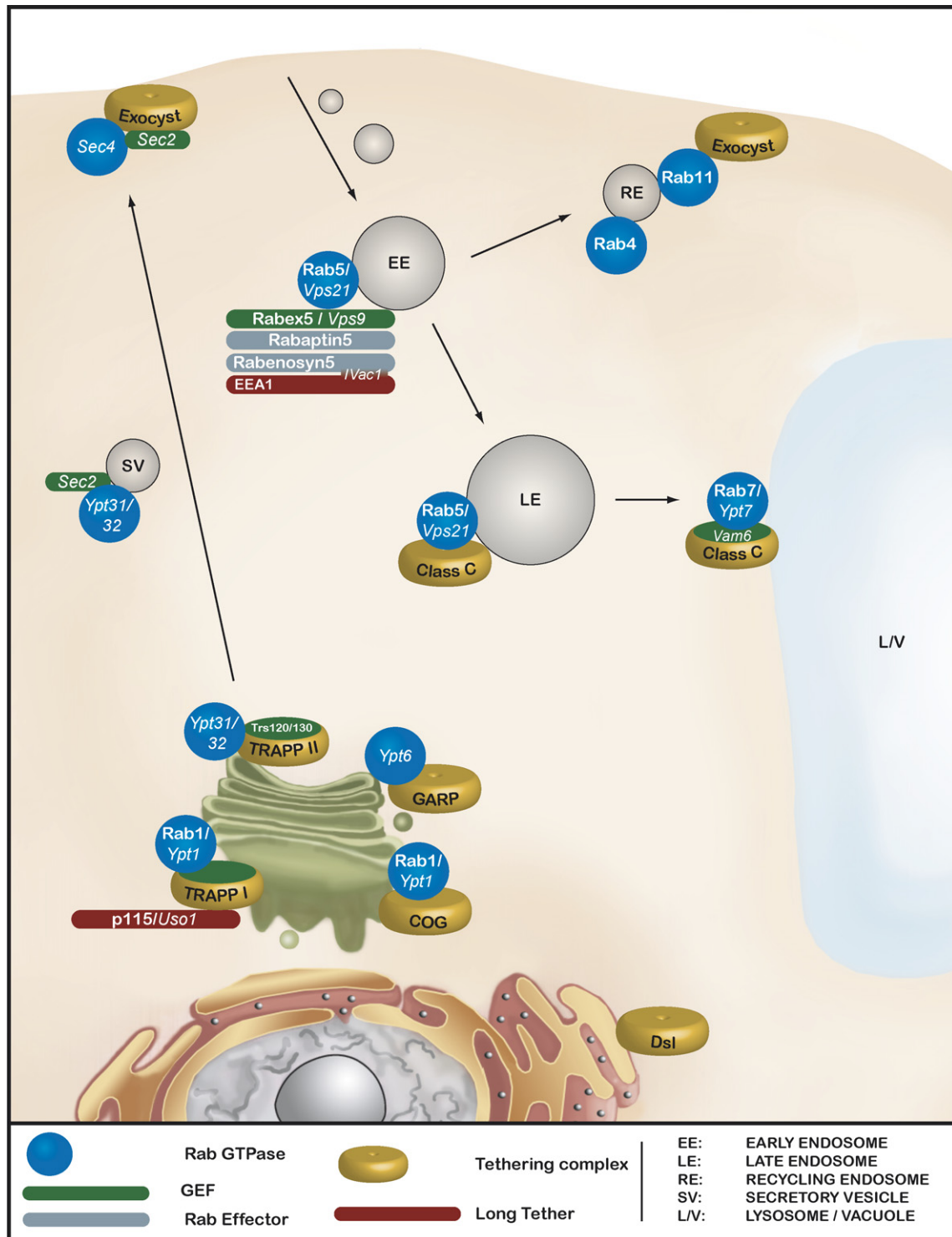


Fig. 2. Intracellular localization of tethers, GEFs and Rabs. The symbols are explained in the figure. Yeast genes are in *italics*. For details see text.

subunits, whereas TRAPP II has two additional subunits (Trs120 and Trs130) and acts as a GEF between late Golgi and endosome [35]. A recent study from the Segev lab showed that TRAPP I is a GEF for Ypt1, whereas TRAPP II activates exclusively Ypt31 [36]. The authors propose that the *cis*-Golgi localized TRAPP I activates the Ypt1 Rab GTPase, which is required for vesicle entry to Golgi apparatus. Indeed, the GEF activity has now been localized to a platform of the

TRAPP I complex, which is composed of several proteins (Bet5–Trs23–Bet3–Trs31) [28]. On the exit side of the Golgi, it is therefore the TRAPP II complex that activates the Ypt31/32 GTPases, which are necessary for the vesicle to be able to leave the Golgi. Since TRAPP II has Trs120 and Trs130 as additional subunits, it is likely that they change the GEF specificity from Ypt1 towards Ypt31/32. Indeed, inactivation of Trs130 leads to mislocalization of Ypt31/32

and enhances the localization of Ypt1 to the Golgi, indicating that the GEF activities are responsible for correct Rab localization [36]. It has been previously suggested that the GEF for the Ypt32 Rab GTPase is a putative effector protein of Ypt1-GTP [37], i.e. Ypt1-GTP could recruit Trs130 and Trs120 to the TRAPPI complex and thereby stimulate activation of Ypt31/32. However, Morozova et al. showed that Ypt1-GTP does not interact with Trs130. It is therefore an open question, how the recruitment of Trs120 and Trs130 is mediated.

Segev and colleagues propose two versions of TRAPPI-II function during Golgi transport. In one, two separate stable TRAPP complexes exist, in another – more dynamic model – the TRAPPII specific subunits Trs120 and Trs130 attach to the TRAPPI complex during Golgi maturation and act as a GEF specificity switch. Either model includes two GEF complexes contributing to the directed flow of secretory cargo through the Golgi apparatus.

2.2. From the TGN to the plasma membrane

Evidence for a Rab-tether cascade from the Golgi to the plasma membrane has been presented. On the secretory vesicles, Ypt32 recruits Sec2, which is a GEF for the Rab Sec4 [38]. Sec2 is an essential hydrophilic protein that is required for the polarized delivery of Sec4 Rab GTPase to exocytic sites. It was shown that Ypt32-GTP recruits Sec2 to exocytic vesicles, which in turn can recruit the Rab Sec4 to drive exocytosis. Interestingly, although Ypt32 and Sec4 share homologous sequence, they bind different sites on the Sec2 protein. In agreement with this observation, only Sec4 is a substrate for Sec2-mediated nucleotide exchange [38]. Sec4-GTP then binds to the exocyst [39], a large eight-subunit complex that tethers vesicles to the plasma membrane. Sec15, an exocyst subunit that is the direct effector of Sec4, could then displace Ypt32-GTP from Sec2, which consequently changes the identity of the exocytic vesicle and prepares it for fusion with the plasma membrane [40]. Because affinity of Sec2 for Sec15 is approximately 3-fold higher than for Ypt32 interaction, the mechanism of Ypt32 displacement is possible *in vivo* [41].

In sum, the connection of Rabs to GEFs and effectors is an underlying principle of secretion from the ER to the plasma membrane. In the following section, we will focus on similar links in the endocytic pathway that became apparent only recently.

3. The Rab-effector network in the endocytic pathway

The endocytic pathway can be dissected into three distinct Rab-specific stages; the (early) endosome (Rab5), the sorting/recycling endosome (Rab4, Rab11), and the lysosome/vacuole as the target organelle for degradation (Rab7). Clathrin-coated-vesicle (CCV) mediated transport in the early endocytic pathway is regulated by Rab5, a GTPase which is required for CCV-endosome and homotypic endosome fusion, whereas recycling processes, during which internalized material is transported back to the plasma membrane, are regulated by Rab4 at the level of the early endosome and Rab11 on recycling endosomes. During recycling, cargo transits through several compartments positive for two Rabs, e.g. Rab5/Rab4 and Rab4/11, indicating that Rab domains on organelles are not static, but merge in a dynamic manner during protein trans-

port [5,42]. These processes depend on a complex network of Rab regulators and effectors. In the case of Rab5, an elaborate, positive feedback-loop, mediated by a GEF-effector complex, is responsible for generating stable, Rab5 positive endosomal structures. Upon membrane recruitment, Rab5 gets activated by the Rabex-5 exchange factor. Rab5-GTP is then able to interact with its effector Rabaptin5, which forms a complex with Rabex-5. Interestingly, the effector stimulates the exchange activity of Rabex-5 on Rab5 [43], thereby recruiting more Rab5 and generating a Rab5 enriched, endosomal domain. Rab5 also recruits phosphoinositide kinases like hVps34, thus promoting binding of effectors with phosphoinositide binding domains like EEA1 and Rabenosyn5, which in turn can promote early endosome fusion [44,45].

3.1. Regulation of endocytic transport

Given the fact that Rab4 and Rab5 are both acting at a similar stage of the endocytic pathway it is likely that mechanisms exist that coordinate the interplay of both GTPases. Similar to the connection of Rabs and GEFs in the early secretory pathway, evidence has been presented that Rab4-GTP also binds to the Rab5 GEF Rabex5 and Rabenosyn5, whereas Rab5-GTP can bind Rab4 effectors [46,47]. This indicates that changes in organelle identity might be driven by a Rab-effector network. In this network, inactivation of the Rabs by GAP proteins seems to be as important as the activation. Recently, Barr and colleagues screened the complete mammalian Rab collection against all GAPs and identified RabGAP-5 as a specific GAP for Rab5 [48]. Consistent with its key function in Rab5 control, inactivation of RabGAP-5 leads to endosomal swelling and a block in transport to the lysosome, whereas overexpression resulted in the loss of EEA1 from endosomes. This indicates that transition through the endocytic pathway is a fine-tuned network controlled by effectors, GEFs and GAPs. It is likely that direct interaction between these factors, as already discovered in some instances, is necessary for coordination.

3.2. Transition between endosome and lysosome

Internalized material destined for degradation diverges from the endosomal recycling route, regulated by Rab4 and 11. Cargo, e.g. epidermal growth factor receptor EGFR, is first internalized into Rab5 positive endosomes and is then transported to late endosomes, carrying Rab7. Finally, proteolysis takes place in lysosomes, which can also be identified by the presence of Rab7. The yeast Rab7 homolog Ypt7 has been shown to be involved in the tethering/docking process of homotypic vacuole fusion and interacts with the vacuolar tethering factor HOPS complex (homotypic vacuole fusion and vacuole protein sorting) in its GTP bound form [18]. The HOPS complex consists of four Class C proteins, Vps11, Vps16, Vps18, and the Sec1p homolog Vps33, and the two additional subunits, Vps39/Vam6 and Vps41/Vam2 [18,19]. Among these subunits, Vps39/Vam6 binds the GDP-bound form of Ypt7 and stimulates nucleotide exchange on this GTPase [19]. Binding of Ypt7-GTP to the HOPS complex suggests that the complex acts as Ypt7 effector, although precise binding studies have to be performed to identify the binding interface between GTPase and effector.

Focusing on the dynamic transport of material destined for degradation leads to the question whether cargo is transferred

from stable Rab5-positive endosomes to stable, Rab7 carrying late endosomes. Alternatively, Rab5 might get dynamically lost from maturing endosomes and could be replaced by Rab7 and its effectors. Zerial and colleagues showed that the endosomes can mature to lysosomes [49]. Using live-cell imaging they demonstrated that Rab5 positive organelles grew in size over time, then lost Rab5 and its effector EEA1, but at the same time acquired Rab7. This Rab-conversion reaction seemed to be mediated by the binding of Rab5 to the lysosomal HOPS complex. Indeed, the authors showed that hVps39 was binding to Rab5-GDP. This would suggest that Rab-exchange is actually driven by a GEF with specificity for Rab5 and Rab7, since yeast Vps39 is a Rab7-specific GEF and does not bind the Rab5 homolog Vps21 [19]. It is therefore likely that additional factors control the specificity of hVps39 to mediate endosome–lysosome transition in mammalian cells. Importantly, these data indicate that a Rab cascade is the basic mechanism for Rab conversion between organelles of the late endocytic pathway. Whether such a transition is also operating in yeast will be an important issue for future studies. The Class C proteins have been shown to interact with another protein, Vps8, at the endosome [50], and Vps8 has been linked to the Rab5 homolog Vps21 [51]. How the Class C complex can operate at two different organelles is still an unresolved issue.

To understand the mechanism by which the Rab conversion process is regulated Roberts et al. investigated phagolysosomal biogenesis [52]. This model system of phagosomes maturing into phagolysosomes mirrors early endosome to lysosome trafficking in the endocytic pathway, especially since it is accompanied by a Rab5–Rab7 conversion. The regulatory key component of Rab conversion and organelle maturation in this system is the small GTPase Rab22a. Rab22a associates with early and late endosomes, but not with lysosomes and overexpression causes morphological enlargement of both compartments [53]. Importantly, overexpression of the GTP-locked mutant Rab22aQ64L prevents maturation of phagosomes indicating a checkpoint function of Rab22a [52]. Consistent with this, GTP-locked mutant Rab22aQ64L caused accumulation of a lysosomal hydrolase, aspartylglucosaminidase, in abnormal vacuole-like structures that contained both, early and late endosome markers. A direct link between Rab22a and Rab-conversion is given by the specific interaction between Rab22a and the Rab5 effector EEA1 [54]. Distinct from Rab5 which binds to both, the N- and the C-terminal regions of EEA1 [13], Rab22a only interacts with the N-terminus suggesting a different function of Rab22a binding.

In summary, the endocytic pathway is regulated by several Rab GTPases, creating different Rab effector-domains, and as in the case of Rab5, even altered membrane composition. Nevertheless it becomes clear that during dynamic trafficking processes, Rab domains get into contact via their effectors, generating directional Rab cascades which can result in Rab conversion accompanying cargo transport and organelle maturation.

4. Concluding remarks

The cumulative recent observations suggest that organelle identity in the secretory pathway is linked to a Rab-effector network. Several examples from the early secretory pathway

and the endocytic pathway highlight that activated Rabs recruit the GEF for the Rab acting in the next trafficking step. Maturation of the organelle is therefore possible and has been demonstrated most convincingly for endosome–lysosome transition. Despite the wealth of information, the precise mechanisms underlying the transition still remain unresolved. It is not clear, when Rabs and tethers are recruited to vesicles or membranes and how this is determined and controlled. It has been shown recently, that TRAPPI interacts with the COPII coat to promote tethering [55]. Potentially, Rabs are already required during vesicle formation. On late endosomes, Rab9 recruits its effector TIP47 already during cargo recruitment [56], and it will be important to test if this principle is conserved. After Rabs are activated by GEFs, how is their half-life on membranes determined? Are GAPs recruited by effector proteins or are they part of the effector network? Are they continuously active or regulated? For the yeast system, we recently showed that the Vam2 subunit of the HOPS tethering complex is phosphorylated by the casein kinase Yck3 [57]. Thus, kinases might control the stability of tethers with Rabs, and promote accessibility of GAPs to the active Rab. With more players in the game, determining the molecular mechanisms underlying Rab function will be an important future task.

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